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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/62, 7/06, A61K 38/28 — A1

(11) International Publication Number: **WO 98/13384**

(43) International Publication Date: 2 April 1998 (02.04.98)

(21) International Application Number: PCT/GB97/02627

(22) International Filing Date: 26 September 1997 (26.09.97)

(30) Priority Data:
9603533-2 27 September 1996 (27.09.96) SE

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: INSULIN C-PEPTIDES

(57) Abstract

The present invention relates to a peptide being a fragment of the human insulin C-peptide, said peptide comprising the sequence ELGGGPGAG or a fragment thereof, or the sequence EGSLQ or a fragment thereof, and having the ability to stimulate Na^+K^+ATPase activity. Further provided are biomimetic organic compounds exhibiting activation of Na^+K^+ATPase activity and/or cellular binding to renal tubule cells and fibroblasts at at least the level exhibited by the aforementioned peptides or their fragments. Such peptides and compounds have utility in combatting diabetes and diabetic complications, or for stimulating Na^+K^+ATPase activity.

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Insulin C-Peptides

The present invention relates to fragments of the insulin C-peptide and their use in the treatment of diabetes and diabetic complications.

Patients with insulin-dependent diabetes mellitus (IDDM), generally synonymous with type 1 diabetes, cannot survive without insulin therapy. IDDM is the classical, life-threatening form of diabetes, the treatment of which was revolutionized by the discovery of insulin in 1922. The prevalence of IDDM in Europe, North America and Japan is 0.25-0.4% of the population. There is a seasonal variation in the incidence of IDDM with more patients presenting in the autumn and winter months. The disorder affects a slight excess of males but this difference becomes less marked with increasing age.

The classical symptoms of IDDM in its acute phase are thirst, large urine volumes, fatigue and weight loss. Less frequent and minor symptoms are muscle cramps, skin infections and blurred vision. Nausea and vomiting may occur in advanced stages and denote impending ketoacidosis and coma. The duration of symptoms is short, usually 2-3 weeks or less. The patients present with high concentrations of glucose and ketone bodies in blood and urine while insulin levels are low or undetectable.

The etiology of IDDM is multifactorial but most likely includes a genetic predisposition for autoimmune reactivity together with environmental triggering, possibly via a virus infection, resulting in partial or complete destruction of the pancreatic beta cells. The

destruction of beta cells may have been in progress during the 6-12 months preceding the onset of the disorder. In the acute phase of IDDM insulin deficiency is thus the dominating pathophysiological feature.

After starting insulin treatment many patients enjoy good blood glucose control with only small doses of insulin. There is an early phase, the "honeymoon period", which may last a few months to a year and which probably reflects a partial recovery of beta cell function. This is, however, a temporary stage and ultimately, the progressive autoimmune destruction of the beta cells leads to increasing requirements for exogenous insulin.

While the short term effects of hypoinsulinemia in the acute phase of IDDM can be well controlled by insulin administration, the long term natural history of IDDM is darkened by the appearance in many patients of potentially serious complications. These include the specifically diabetic problems of nephropathy, retinopathy and neuropathy. These conditions are often referred to as microvascular complications even though microvascular alterations are not the only cause. Atherosclerotic disease of the large arteries, particularly the coronary arteries and the arteries of the lower extremities, may also occur.

Nephropathy develops in approximately 35% of IDDM patients particularly in male patients and in those with onset of the disease before the age of 15 years. The diabetic nephropathy is characterized by persistent albuminuria secondary to glomerular capillary damage, a progressive reduction of the glomerular filtration rate and eventually, end stage renal failure.

The prevalence of diabetic retinopathy is highest among young-onset IDDM patients and it increases with the duration of the disease. Proliferative retinopathy is generally present in about 25% of the patients after 15 years duration and in over 50% after 20 years. The earliest lesion of diabetic retinopathy is a thickening of the capillary basement membrane, there is then capillary dilatation and leakage and formation of microaneurysms. Subsequently, occlusion of retinal vessels occurs resulting in hypoperfusion of parts of the retina, oedema, bleeding and formation of new vessels as well as progressive loss of vision.

Diabetic neuropathy includes a wide variety of disturbances of somatic and autonomic nervous function. Sensory neuropathy may cause progressive loss of sensation or, alternatively, result in unpleasant sensations, often pain, in the legs or feet. Motor neuropathy is usually accompanied by muscle wasting and weakness. Nerve biopsies generally show axonal degeneration, demyelination and abnormalities of the vasa nervorum. Neurophysiological studies indicate reduced motor and sensory nerve conduction velocities. Autonomic neuropathy afflicts some 40% of the patients with IDDM of more than 15 years duration. It may evolve through defects in thermoregulation, impotence and bladder dysfunction followed by cardiovascular reflex abnormalities. Late manifestations may include generalized sweating disorders, postural hypotension, gastrointestinal problems and reduced awareness of hypoglycemia. The latter symptom has grave clinical implications.

Several theories have been advanced with regard to possible mechanism(s) involved in the pathogenesis of the different diabetic complications (1). Metabolic factors may be of importance and recent studies

demonstrate that good metabolic control is accompanied by significantly reduced incidence of complications of all types (2). Nevertheless, after 7-10 years of good metabolic control as many as 15-25% of the patients show signs of beginning nephropathy, 10-25% have symptoms of retinopathy and 15-20% show delayed nerve conduction velocity indicating neuropathy. With longer duration of the disease the incidence of complications increases further.

C-peptide is a part of the proinsulin molecule which, in turn, is a precursor to insulin formed in the beta cells of the pancreas. Human C-peptide is a 31 amino acid peptide having the following sequence: EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ (SED ID. NO. 1). It has been suggested in EP 132 769 that C-peptide may be given for the treatment of diabetes and in SE 460334 that insulin in combination with C-peptide can be administered in the treatment of diabetes and in the prevention of diabetic complications.

In recent years it has become apparent that type 1 diabetes is accompanied by consistently reduced activity of the enzyme Na^+K^+ATPase in several tissues, notably in renal glomeruli, retina, peripheral nerve, heart and skeletal muscle (3, 4, 5). Na^+K^+ATPase is an enzyme that is localized to the cell membrane and generates energy for transcellular transport of Na^+ and K^+ as well as for all co- or countertransported substrates in all mammalian cells. It is thus obvious that the activity of this enzyme is of fundamental importance for normal cell function. Deficient Na^+K^+ATPase activity in nervous tissue, glomeruli and retina is likely to be an important contributing factor in the pathogenesis of diabetic neuropathy, nephropathy and retinopathy. Na^+K^+ATPase activity is regulated via the Na^+ concentration and by hormonal action; several hormones

stimulate (thyroid hormone, noradrenalin, angiotensin, neuropeptide Y, insulin) or inhibit (dopamine, ANF) the enzyme's activity (6). Despite insulin treatment sufficient to achieve good glycemic control, patients with type 1 diabetes show signs of insufficient Na^+K^+ATPase activity on a long term basis.

The present invention is based on the discovery of a group of peptides from the middle portion and the C-terminal part of the C-peptide molecule which are characterized by a remarkable ability to stimulate Na^+K^+ATPase activity. These peptides are all small fragments of the C-peptide molecule. C-peptide itself is able to stimulate Na^+K^+ATPase via activation of a G-protein, increase in the intracellular Ca^{2+} concentration and activation of protein phosphatase 2B (7). However, the smaller peptides' stimulatory effect on Na^+K^+ATPase activity is similar to or greater than that of C-peptide itself. There is both *in vitro* and in *vivo* evidence to indicate that upon administration of one of these peptides together with regular insulin treatment, renal function improves, early signs of retinopathy regress and the function of somatic and autonomic nerves improves. Treatment with these specific peptides, optionally in combination with conventional insulin therapy is thus useful in preventing or substantially retarding the development of late diabetic complications. A potential advantage that the small peptides possess over C-peptide is that they may be administered orally instead of by injection as in the case of C-peptide and insulin.

In one aspect, the present invention thus provides a peptide being a fragment of the human insulin C-peptide, said peptide comprising the sequence ELGGGPGAG (SEQ ID NO. 2) (hereinafter "peptide A") or a fragment thereof, or the sequence EGSLQ (SED ID NO. 3) (hereinafter

"peptide E"), or a fragment thereof, and having the ability to stimulate Na^+K^+ATPase activity.

In a more particular embodiment, the present invention provides a peptide having the sequence ELGGGPGAG (SEQ ID NO. 2) or EGSLQ (SEQ ID NO. 3), or a fragment thereof.

Especially, the invention provides such peptides for use in therapy and more particularly for use in combatting diabetes and diabetic complications.

In another aspect the present invention provides a pharmaceutical composition comprising a peptide of the invention or a fragment thereof as hereinbefore defined, together with at least one pharmaceutically acceptable carrier or excipient.

A yet further aspect of the present invention provides the use of a peptide of the invention, or a fragment thereof, as hereinbefore defined, in the manufacture of a medicament for combatting diabetes or diabetic complications.

As used herein the term "combatting" includes both treatment and prophylaxis.

The present invention thus relates to the use of the following peptides which all are fragments of C-peptide: Peptide A (amino acid sequence ELGGGPGAG)(SEQ ID NO. 2) or components thereof, for example Peptide B (ELGG)(SEQ ID NO. 4), Peptide C (ELGGGP)(SEQ ID NO. 5) or Peptide D (GGPGA)(SEQ ID NO. 6). In addition, the invention includes Peptide E (EGSLQ)(SEQ ID NO. 3) and parts thereof, for example Peptide F (GSLQ)(SEQ ID NO. 7). All are intended for the manufacture of a medicament for treating type 1 diabetes.

Fragments of the invention have been proven to stimulate Na^+K^+ATPase activity to varying extent. Thus, studies involving renal tubule cells under *in vitro* conditions indicate that Peptides A-D stimulate Na^+K^+ATPase activity to an extent comparable to that for the whole C-peptide molecule. As much as 90% of the effect is achieved within 3 minutes. Moreover, Peptides E and F possess a stimulatory effect on Na^+K^+ATPase of renal cells which is comparable to or greater than that for the whole molecule. Combinations of Peptides A-D with Peptides E or F result in a stimulation of the enzyme activity that is greater than that for either peptide alone. For detailed examples of the stimulatory effects of the above peptides, see Example 1, below.

C-peptide exhibits specific binding to the surface of several cell types, notably renal tubule cells and fibroblasts. When fluorescently labelled C-peptide is incubated with cells it binds to the cell surface. The specificity of the binding is illustrated by the fact that preincubation with unmarked C-peptide prevents binding of the fluorescently labelled C-peptide. When preincubation with the fragments of the invention, particularly with either of fragments E or F was made, the fragments were found to prevent binding of the fluorescently marked C-peptide, demonstrating that the fragments bind specifically to the same binding site on the cell surface as C-peptide itself. For a detailed example of the binding of Fragment E see Example 28, below.

As mentioned above, included within the scope of the invention are peptides comprising the sequences of not only peptides A and E, but also their fragments. In the case of the nonapeptide A, such fragments may be 8 to 2 amino acids in length. In the case of the pentapeptide peptide E, such fragments may be 4 to 2 amino acids in

length. Exemplary fragments B, C and D (for peptide A) and F (for peptide E) are listed above, but other fragments are also included.

In the case of peptide A certain studies on Na^+K^+ATPase activity, studying the ability of the peptide fragments to stimulate the activity of Na^+K^+ATPase of rat renal tubule segments, have shown that one or more of the central tri-glycine residues may be important, and preferred peptide fragments, where peptide A is concerned, thus include at least one, and more preferably, at least two, of the central tri-glycine residues. Thus, in addition to peptides B, C and D mentioned above, representative exemplary peptide fragments include GGGPGAG (SEQ ID NO. 8), GGGPG (SEQ ID NO. 9), GGGP (SEQ ID NO. 10), GGP and GGPG (SEQ ID NO. 11).

Furthermore, it has been found that peptides containing non-natural D-amino acid isomers may also be active, including for example the dipeptide D-LG or D,L-LG. Thus, included within the scope of the invention are "non-native" isomers of the "native" L-amino acid C-peptide sequences. Insofar as peptide A is concerned, it is believed that the presence of at least one (if D-peptide) or two (if L-peptide) of the central tri-glycine residues may be important in a 9 amino acid or less peptide segment.

In the case of peptide E, exemplary representative fragments include not only the tetrapeptide, peptide F, but also SLQ and LQ. The C-terminal Q residue is believed to be of importance. Likewise, non-native isomers or derivatives of the peptides e.g. peptides including D-amino acids are included within the scope of the invention.

The invention encompasses peptides comprising the sequences of peptides A and E. Thus, also included within the scope of the invention are peptides having N- and/or C-terminal extensions, or flanking sequences, to the sequences of peptides A and C. Such peptides may include additional amino acids which may either be those provided in the corresponding position in the native human insulion C-peptide or other amino acids (excluding of course the possibility of reconstituting the entire insulin C-peptide). The length of such "extended" peptides may vary, but preferably the peptides of the invention are no more than 25 or 20, especially preferably not more than 15 or 10 amino acids in length. Exemplary peptides include octa-, hepta and hexa-peptides including the sequence of peptide E, e.g. LALEGSLQ (SEQ ID NO. 12), ALEGSLQ (SEQ ID NO. 13) and LEGSLQ (SEQ ID NO. 14).

The peptides of the invention can be used for the treatment of diabetes and diabetic complications, most notably type 1 diabetes and its complications. As used herein the term "diabetic complications" thus includes all complications known in the art to be associated with various forms of diabetes. Whilst not wishing to be bound by theory, the utility of the peptides is believed, as explained above, to be linked to their ability to stimulate Na^+K^+ATPase activity. A further aspect of the invention thus includes the peptides for use in, and their use in preparing medicaments for use in stimulating Na^+K^+ATPase activity in a subject.

Na^+K^+ATPase activity may readily be assayed using techniques known in the art and described in the literature and thus the effect of the peptides in stimulating Na^+K^+ATPase activity may readily be determined (for example, see reference 7).

Thus, the peptides can be used for the manufacture of a medicament for stimulation of Na^+K^+ATPase activity, for treating type 1 diabetes patients with retinopathy, for treating type 1 diabetes patients with nephropathy, for treating type 1 diabetes patients with neuropathy and for retarding the development of late diabetic complications. The medicament may comprise insulin. The invention also relates to the method for treatment or prevention of the above given indications.

The peptides of the invention may be used singly or in combination and thus a pharmaceutical composition or medicament may be prepared comprising one or more of the peptides. As mentioned above, a synergy has been observed between peptide A or peptides based on or derived from peptide A (the "peptide A group") and peptide E or peptides based on or derived from peptide E (the "peptide E group"). Thus, synergistic combinations of a peptide from the peptide A group, with a peptide from the peptide E group represent a preferred embodiment of the invention.

The peptides may also be used in combination or conjunction with other agents active or effective to treat diabetes and/or its complications. Such other active agents include for exammple insulin. In such "combination" therapies the peptide(s) and second active agent may be administered together in the same composition or separately in separate compositions, simultaneously or sequentially.

A further aspect of the invention thus provides a product containing a peptide of the invention, or a fragment thereof, as hereinbefore defined together with a further active agent effective to combat diabetes or diabetic complications, as a combined preparation for simultaneous, separate or sequential use in combatting

diabetes and/or diabetic complications. Preferably such a further active agent is insulin.

In such combined therapies, where insulin is used, it is to be understood that the term "insulin" encompasses all forms, types and derivatives of insulin which may be used for therapy e.g. synthetic, modified, or truncated variants of the active human insulin sequence.

The compositions of the invention may be administered orally or parenterally by the subcutaneous, intramuscular or intravenous route. The compositions of this invention comprise active fragments/peptides of the C-peptide molecule (e.g. Peptides A-F), together with a pharmaceutically acceptable carrier therefor and optionally, other therapeutic ingredients, for example human insulin. The total amount of active ingredients in the composition varies from 99.99 to 0.01 percent of weight. The carrier must be acceptable in the sense that it is compatible with other components of the composition and is not deleterious to the recipient thereof.

The compositions may be formulated according to techniques and procedures well known in the art and widely described in the literature, and may comprise any of the known carriers, diluents or excipients. Thus, for example, compositions of this invention suitable for parenteral administration conveniently comprise sterile aqueous solutions and/or suspensions of the pharmaceutically active ingredients (e.g. Peptides A-F) preferably made isotonic with the blood of the recipient, generally using sodium chloride, glycerin, glucose, mannitol, sorbitol, and the like. In addition, the compositions may contain any of a number of adjuvants, such as buffers, preservatives, dispersing agents, agents that promote rapid onset of action or

prolonged duration of action and the like.

Compositions of this invention suitable for oral administration may, for example, comprise active fragments/peptides of the C-peptide molecule (e.g. Peptides A-F) in sterile purified stock powder form preferably covered by an envelope or envelopes (enterocapsule) protecting from degradation (decarboxylation or hydrolysis) of the active peptides in the stomach and thereby enabling absorption of these substances from the gingiva or in the small intestine. The envelope(s) may contain any of a number of adjuvants such as buffers, preservative agents, agents that promote prolonged or rapid release giving an optimal bioavailability of the compositions in this invention, and the like.

In addition, the present invention relates to non-peptide compounds showing the same stimulatory effects as displayed by their C-peptide-derived counterparts. Such peptidomimetics or "small-molecules" capable of mimicking the activity of the naturally occurring proteins or peptides are likely to be better suited for e.g. oral delivery due to their increased chemical stability (8,9).

It is now commonplace in the art to replace peptide or protein-based active agents e.g. therapeutic peptides with such peptidomimetics having functionally-equivalent activity. Various molecular libraries and combinatorial chemistry techiques exist and are available to facilitate the identification, selection and/or synthesis of such compounds using standard techniques (10). Such standard techniques may be used to obtain the peptidomimetic compounds according to the present invention, namely peptidomimetic organic compounds which show substantially similar or the same activation of

$Na^+K^+ATPase$ and/or cellular binding characteristics as the peptides of the invention, e.g. as described herein in the Examples.

A further aspect of the invention thus provides a biomimetic organic compound based on the peptides of the invention, characterised in that said compound exhibits activation of $Na^+K^+ATPase$ and/or cellular binding characteristics to renal tubule cells and fibroblasts at at least the level exhibited by the peptides and peptide fragments of the invention as hereinbefore defined.

The invention will now be described in more detail in the following non-limiting Examples which show, *inter alia*, the stimulatory effect of specific peptides on $Na^+K^+ATPase$ activity, and cell-binding with reference to the drawing in which:

Figure 1 shows a chromatogram from a preparative reverse phase purification of human C-peptide labelled with tetramethylrhodamine. The column was eluted with a 20 to 40% acetonitrile gradient (acetonitrile in 0.1% trifluoroacetic acid (TFA) during 20 minutes. Peak A corresponds to unreacted fraction of C-peptide. Peaks B and C correspond to C-peptide labelled with tetramethyl-rhodamine. The separation of the B and C peaks corresponds to the presence of two tetramethylrhodamie isomers in the activated reagent. For further studies material from the C-peak was used. Solid line corresponds to absorbtivity at 220 nm (peptide) and dashed line to absorbtivity at 555 nm (tetramethylrhodamine).

Example 1

The stimulatory effect of Peptides A-F on $Na^+K^+ATPase$ activity of rat renal tubule cells was examined. Single proximal convoluted tubules were prepared from rat

kidneys by micro-dissection. The tubules were incubated for 30 minutes at room temperature with either of Peptides A-F or rat C-peptide 1. Na^+K^+ATPase activity was then measured following exposure of the tubules to hypotonic shock and incubation for 15 minutes in a medium containing ^{32}P-ATP in the presence or absence of oubain.

The stimulatory activity of $5\text{-}10^{-7}$ M rat C-peptide 1 was set at 100%. For the same concentration of Peptides A-F the following relative activities were obtained:

Peptide A 88±3 percent
Peptide B 36±2 percent
Peptide C 46±3 percent
Peptide D 65±4 percent
Peptide E 110±3 percent
Peptide F 96±2 percent
Peptides B + C 86±3 percent

Examples of particular pharmaceutical compositions of this invention are provided in the examples below.

Example 2

Human Insulin: Peptide A alone or in equimolar mixture with Peptides B, C, D, E and F (1:4 on a molar basis at 100Units M insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin (28U/mg) - 1000U
Peptide A alone - 16.8 mg
M-Kreosol - 25 mg
Glycerol - 160 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide sufficient to make a composition vloume of 10 ml and a final pH of 7.0-7.8 or a combination with

Peptide A - 16.8 mg
Peptide B - 8.8 mg
Peptide C - 13.6 mg
Peptide D - 10 mg
Peptide E -12.4 mg
Peptide F - 9.2 mg
M-Kresol - 25 mg
Glycerol - 160 mg

Water and either 10% - hydrochloride acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.0-7.8

Example 3

Human Insulin: Peptide B (1:4 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin (28U/mg) - 1000U
Peptide B - 8.8 mg
M-Kresol - 25 mg
Glycerol - 160 mg

Water and either 10% hydrochloride acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.7-7.8

Example 4

Human Insulin: Peptide C (1:4 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin (28U/mg) - 1000U
Peptide C - 13.6 mg
M-Kresol - 25 mg
Glycerol - 160 mg

Water and either 10% hydrochloride acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.0-7.8

Example 5

Human Insulin: Peptides D (1:5 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin (28U/mg) - 1000U
Peptide D - 10.0 mg
M-kreosol - 25 mg
Glycerol - 160 mg

Water and either 10% hydrochloride acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.0-7.8

Example 6

Human Insulin: Peptide E (1:4 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin (28U/mg) - 1000U
Peptide E - 12.4 mg
M-Kresol - 25 mg
Glycerol - 160 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.0-7.8

Example 7

Human Insulin: Peptide E (1:4 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix

Human Insulin (28U/mg) - 1000U
Peptides F - 9.2 mg
M-Kreosol - 25 ml
Glycerol - 160 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.0-7.8

Example 8

Human Insulin: Peptide A alone or mixed equimolar together with fragments B, C, D, E and F (1:1 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin (28U/mg) - 1000U
Peptide A - 4.2 mg
M-Kreosol - 25 mg
Glycerol - 160 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.0-7.8

or a combination with
Peptide A - 4.2 mg
Peptide B - 2.2 mg
Peptide C - 3.4 mg
Peptide D - 2.5 mg
Peptide E - 3.1 mg
Peptide F - 2.3 mg
M-Kreosol - 25 mg
Glycerol - 160 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.0-7.8

Example 9

Human Insulin: Peptide B (1:1 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin (28U/mg) - 1000U
Peptide B - 2.2 mg
M-Kreosol - 25 mg
Glycerol - 160 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.0-7.8

Example 10

Human Insulin: Peptide C (1:1 on a molar basis at 100Units (U) insulin per ml).
To prepare 10 ml of the composition, mix
Human Insulin (28U/mg) - 1000U
Peptide C - 3.4 mg
M-Kieosol - 25 ml
Glycerol - 160 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.0-7.8

Example 11

Human Insulin: Peptide D (1:1 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin (28U/mg) - 1000U
Peptide D - 2.5 mg
M-Kreosol - 25 mg
Glycerol - 160 mg

Water and either 10% hydrochloric acid of 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.0-7.8

Example 12

Human Insulin: Peptide E (1:1 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin (28U/mg) - 1000U
Peptide E - 3.1 mg
M-Kreosol - 25 mg
Glycerol - 160 ml

Water and either 10% hydrochloric acid of 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.0-7.8

Example 13

Human Insulin: Peptide E (1:1 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin (28U/mg) - 1000U
Peptide F - 2.3 mg
M-Kreosol - 25 mg
Glycerol - 160 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.0-7.8

Example 14

Human Zinc Insulin: Peptide A alone or mixed equimolar together with fragments B, C, D, E and F (1:4 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin in modific amorph 300 U and modific cryst 700 U (28U/mg) - 1000 U
Peptide A - 16.8 mg
Zinc - 1.3 mg
Sodium chloride - 70 mg
Sodium Acetate - 16 mg
Methyl Parahydroxybenz - 10 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.1-7.4

or a combination with
Peptide A - 16.8 mg
Peptide B - 8.8 mg
Peptide C - 13.6 mg
Peptide D - 10 mg
Peptide E - 12.4 mg
Peptide F - 9.2 mg
Zinc - 1.3 mg
Sodium chloride - 70 mg
Sodium Acetate - 16 mg
Methyl Parahydroxybenz - 10 mg
Water and either 10% hydrochloric sodium hydroxide sufficient to make a composition volume and a final pH of 7.1-7.4

Example 15

Human Zinc Insulin: Peptide B (1:4 on a molar basis at 100Units (U) insulin per ml).
To prepare 10 ml of the composition, mix
Human Insulin in modific amorph 300U and modific cryst 700U
(28U/mg) - 1000U
Peptide B - 8.8 mg
Zinc - 1.3 mg

Sodium chloride - 70 mg
Sodium Acetate - 16 mg
Methyl Parahydroxybenz - 10 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide suficient to make a composition volume of 10 ml and a final pH of 7.1-7.4

Example 16

Human Zinc Insulin: Peptide C (1:4 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin in modific amorph 300U and modific cryst 700U
(28U/mg) - 1000U
Peptide C - 13.6 mg
Zinc - 1.3 mg
Sodium chloride - 70 mg
Sodium Acetate - 16 mg
Methyl Parahydroxybenz - 10 mg

Water and either 10% hyrochloric acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.1-7.4

Example 17

Human Zinc Insulin: Peptide D (1:4 on a molar basis at 100Units (U) insulin per ml).
To prepare 10 ml of the composition, mix
Human Insulin in modific amorph 300U and modific cryst 700U
(28U/mg) - 1000U
Peptide D - 10.0 mg
Zinc- 1.3mg
Sodium chloride - 70 mg
Sodium Acetate - 16 mg

Methyl Parahydroxybenz - 10 mg

Water and either 10% hydrochloric acid of 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.1-7.4

Example 18

Human Zinc Insulin: Peptide E (1:4 on a molar basis at 100Units (U) insulin per ml).
To prepare 10 ml of the composition, mix
Human Insulin in modific amorph 300 and modific cryst 700U
(28U/mg) - 1000U
Peptide E - 12.4 mg
Zinc - 1.3 mg
Sodium chloride - 70 mg
Sodium Acetate -16 mg
Methyl Parahydroxybenz - 10mg

Water and either 10% hydrochloric acid of 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.1-7.4

Example 19

Human Zinc Insulin: Peptide F (1:4 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin in modific amorph 300U and modific cryst 700U
(28U/mg) - 1000U
Peptide F - 9.2 mg
Zinc - 1.3 mg
Sodium chloride - 70 mg
Sodium Acetate - 16 mg
Methyl Parahydroxybenz - 10 mg

Water and either 10% hydrochloric acid of 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.1-7.4

Example 20

Human Zinc insulin: Peptide A alone or mixed equimolar together with fragments B, C, D, E and F (1:1 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin in modific amorph 300U and modific cryst 700U
(28U/mg) - 1000U
Peptide A - 4.2 mg
Zinc - 1.3 mg
Sodium chloride - 70 mg
Sodium Acetate - 16 mg
Methyl Parahydroxybenz - 10 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.1-7.4

or a combination with
Peptide A - 4.2 mg
Peptide B - 2.2 mg
Peptide C - 3.4 mg
Peptide D - 2.5 mg
Peptide E - 3.1 mg
Peptide F - 2.3 mg
Zinc - 1.3 mg
Sodium chloride - 70 mg
Sodium Acetate - 16 mg
Methyl Parahydroxybenz - 10 mg

Water and either 10% hydrochloric acid of 10% sodium hudroxide suffient to make a composition volume of 10 ml

and a final pH of 7.1-7.4

Example 21

Human Zinc Insulin: Peptide B (1:1 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin in modific amorph 300U and modific cryst 700U
(28U/mg) - 1000U
Peptide B - 2.2 mg
Zinc - 1.3 mg
Sodium chloride - 70 mg
Sodium Acetate - 16 mg
Methyl Parahydroxybenz - 10 mg

Water and either 10% hydrochloric acid of 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.1-7.4

Example 22

Human Zinc Insulin: Peptide C (1:1 on a molar basis at 100Units (U) insulin per ml).
To prepare 10 ml of the composition, mix
Human Insulin in modific amorph 300U and modific cryst 700U
(28U/mg) - 1000U
Peptide C - 3.4 mg
Zinc - 1.3 mg
Sodium chloride - 70 mg
Sodium Acetate - 16 mg
Methyl Parahydroxybenz - 10 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.1-7.4

Example 23

Human Zinc Insulin: Peptide D (1:1 on a molar basis at 100Units (U) insulin per ml).

Human Insulin in modific amorph 300U and modific cryst 700U
(28U/mg) - 1000 U
Peptide D - 2.5 mg
Zinc - 1.3mg
Sodium chloride - 70 mg
Sodium Acetate - 16 mg
Methyl Parahydroxybenz - 10 mg

Water and either 10% hydrochloric acid or 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.1-7.4

Example 24

Human Zinc Insulin: Peptide E (1:1 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin in modific amorph 300U and modific cryst 700U
(28U/mg) - 1000U
Peptide E - 3.1 mg
Zinc - 1.3 mg
Sodium chloride - 70 mg
Sodium Acetate - 16 mg
Methyl Parahydroxybenz - 10 mg

Water and either 10% hydrochloric acid or 10 sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.1-7.4

Example 25

Human Zinc Insulin: Peptide F (1:1 on a molar basis at 100Units (U) insulin per ml).

To prepare 10 ml of the composition, mix
Human Insulin in modific amorph 300U and modific cryst 700U
(20U/mg) - 1000U
Human Insulin in modific amorph 300U and modific cryst 700U
(28U/mg) - 1000U
Peptide F - 2.3 mg
Zinc - 1.3 mg
Sodium chloride - 70 mg
Sodium Acetate-16mg
Methyl Parahydroxybenz - 10 mg

Water and either 10% hydrochloric acid of 10% sodium hydroxide sufficient to make a composition volume of 10 ml and a final pH of 7.1-7.4

Example 26

Peptide A
To prepare sublingual tablets of enterocapsules each containing the composition equimolar to 100U of insulin, mix
Peptide A - 0.42 mg
Lactos - 30 mg
et const q s
or in combination of Peptide A: Peptide B: Peptide C: Peptide D: Peptide E: Peptide F: (1:1:1:1:1:1 on molar basis)

To prepare sublingual tablets of enterocapsulas each containing the composition equimolar to 100U of insulin, mix
Peptide A - 0.42 mg

Peptide B - 0.22 mg
Peptide C - 0.34 mg
Peptide D - 0.25 mg
Peptide E - 0.31 mg
Peptide F - 0.23 mg
Lactos - 30 mg
et const q s

Example 27

Peptide A
To prepare sublingual tablets or enterocaplulas each containing the composition equimolar to 400U of insulin, mix
Peptide A - 1.67 mg
Lactos - 30 mg
et const q s
or in combination of Peptide A: Peptide B: Peptide C: Peptide D: Peptide E: Peptide F (1:1:1:1:1:1 on molar basis)
To prepare sublingual tablets or enterocapsulas each containing the composition equimolar to 400U of insulin, mix
Peptide A - 1.68 mg
Peptide B - 0.88 mg
Peptide C - 1.36 mg
Peptide D - 1.0 mg
Peptide E - 1.24 mg
Peptide F - 0.92 mg
Lactos - 30 mg
et const q s

Example 28

The specific binding of Peptide E to the cell surface is illustrated as follows. Human biosynthetic C-peptide (Eli-Lilly, Inc., Indianapolis, USA) was labelled with tetramethylrhodamine using the activated reagent tetramethylrhodamine succinimidyl ester (FluoReporter®

Protein labelling kit, Art. no. F-6163; Molecular Probes Europe BV, Leiden, Netherlands). The coupling reaction was performed at pH 8.3 (0.1 M $NaHCO_3$ buffer) with a five-fold stoichiometrical excess of activated reagent to C-peptide. The tetramethylrhodamine group has absorption/emission maxima at 555/580 nm, respectively and is incorporated in the N-terminus of the C-peptide. Labelled C-peptides were purified by gel filtration (desalting against 50 mM phosphate buffer, 0.1 M NaCl, pH 7.4) on a NAP-5 column; Pharmacia Biotech Uppsala, Sweden) and subsequently by preparative reverse phase chromatography (250 mm Kromasil C8 column, diam. 4.6 mm, 7 μm particle size, 10 nm pore size, Eka-Nobel, Surte, Sweden) using a 1090 Hewlett Packard HPLC chromatography system (Grenoble, France) (Fig. 1). Eluted material was immediately adjusted to pH 8 by addition of ammonia and subsequently lyophilized.

Cultured human renal tubule cells (proximal convoluted tubules, PCT) were incubated with the rhodamine labelled C-peptide synthesized as described above. The cells were prepared from the healthy part of a human kidney removed surgically because of hypernephroma. The outer 150 μm of the renal cortex was removed in a microtome and incubated in a collagenase solution (0.05%) at 37°C for 15 minutes. A tissue suspension was centrifuged and rinsed twice with 0.01% soybean trypsin inhibitor-solution (Gibco Laboratories, Grand Island, N.Y., USA) and a concentrate of PCT fragments and PCT cells were plated onto glass cover slips. The cells were cultured in Dulbecco's Modified Eagle's Medium [DMEM, 20 mmol/l 4-(2-hydroxyethyl)-1-piperazineethane sulphoric acid (Hepes), 24 mmol/l $NaHCO_3$ 50,000 IU/l penicillin and 50 mg/l streptomycin, pH 7.4] with 10% fetal bovine serum (Gibco) in an incubator at 37°C with 95% O_2, and 5% CO_2. After 28 hours in culture the medium was changed to DMEM with 1% fetal bovine serum. The cells were examined

approximately 18-36 hours later.

The interaction between C-peptide and the cell surface of the tubule cells was recorded using fluorescence correlation spectroscopy (11). Using a C-peptide concentration of 5 nM 92% of the peptide was found to be bound to the cell surface within 50 minutes. In contrast, when the cells were preincubated with 5 μM of Peptide E, C-peptide binding after 50 minutes was no more than 12%. Likewise, when C-peptide had been bound to the cells for 50 minutes and Peptide E was added afterwards, this resulted in dislocation of a major proportion of the C-peptide from its binding site within 4 hours; only 14% remained bound. Similar conditions obtained for peptide F. The results indicate that the peptides - in similarity to C-peptide - bind to a specific binding site on the cell surface.

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SEQUENCE LISTING

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(ii) TITLE OF INVENTION: Insulin C-peptides

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: SE 96035533.2

(B) FILING DATE: 27-SEP-1996

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro
1 5 10 15

Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln
20 25 30

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Glu Leu Gly Gly Gly Pro Gly Ala Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Glu Gly Ser Leu Gln
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Glu Leu Gly Gly
1

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Glu Leu Gly Gly Gly Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Gly Gly Pro Gly Ala
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Gly Ser Leu Gln
1

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Gly Gly Gly Pro Gly Ala Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Gly Gly Gly Pro Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Gly Gly Gly Pro
1

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Gly Gly Pro Gly
1

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Leu Ala Leu Glu Gly Ser Leu Gln
1 5

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Ala Leu Glu Gly Ser Leu Gln
1 5

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Leu Glu Gly Ser Leu Gln
1 5

Claims

1. A peptide being a fragment of the human insulin C-peptide, said peptide comprising the sequence ELGGGPGAG (SEQ ID NO. 2) or a fragment thereof, or the sequence EGSLQ (SEQ ID NO. 3) or a fragment thereof, and having the ability to stimulate Na^+K^+ATPase activity.

2. A peptide as claimed in claim 1 having the sequence ELGGGPGAG (SEQ ID NO. 2) or EGSLQ (SEQ ID NO. 3), or a fragment thereof.

3. A peptide as claimed in claim 2 wherein said fragment is selected from: ELGG (SEQ ID NO. 4) (peptide B), ELGGGP (SEQ ID NO. 5) (peptide C), GGPGA (SEQ ID NO. 6) (peptide D) and GSLQ (SEQ ID NO. 7) (peptide F).

4. A peptide or fragment as claimed in claim 1 or claim 2 which is 2 to 25 amino acids in length.

5. A peptide or fragment as claimed in claim 4 being 2 to 9 amino acids in length.

6. A biomimetic organic compound characterised in that said compound exhibits activation of Na^+K^+ATPase and/or cellular binding characteristics to renal tubule cells and fibroblasts at at least the level exhibited by a peptide or fragment as defined in any one of claims 1 to 5.

7. A peptide or fragment as claimed in any one of claims 1 to 5 or a biomimetic organic compound as claimed in claim 6 for use in therapy.

8. A peptide or fragment thereof or biomimetic organic compound thereof as claimed in claim 7 for use in combatting diabetes and/or diabetic complications, or for

stimulating Na^+K^+ATPase activity.

9. A pharmaceutical composition comprising a peptide or fragment or biomimetic organic compound thereof as claimed in any one of claims 1 to 8 together with at least one pharmaceutically acceptable carrier or excipient.

10. A pharmaceutical composition as claimed in claim 9 further comprising at least one additional active agent effective to combat diabetes or diabetic complications.

11. A pharmaceutical composition as claimed in claim 10 wherein said additional active agent is insulin.

12. Use of a peptide or fragment thereof or biomimetic organic compound thereof as defined in any one of claims 1 to 6 for preparing a medicament for combatting diabetes and diabetic complications, or for stimulating Na^+K^+ATPase activity.

13. Use as claimed in claim 12 further comprising the use of insulin.

14. Use as claimed in claim 12 or claim 13 wherein said medicament is used for treating type 1 diabetes, optionally with nephropathy, neuropathy or retinopathy or for retarding the development of late diabetic complications.

15. A product containing a peptide, or fragment or biomimetic organic compound thereof as defined in any one of claims 1 to 6 together with at least one additional active agent effective to combat diabetes or diabetic complications as a combined preparation for simultaneous, separate or sequential use in combatting diabetes and/or diabetic complications.

16. A method of combatting diabetes or diabetic complications in a human or non-human subject comprising administering to said subject a peptide or fragment or biomimetic organic compound thereof as claimed in any one of claims 1 to 6.

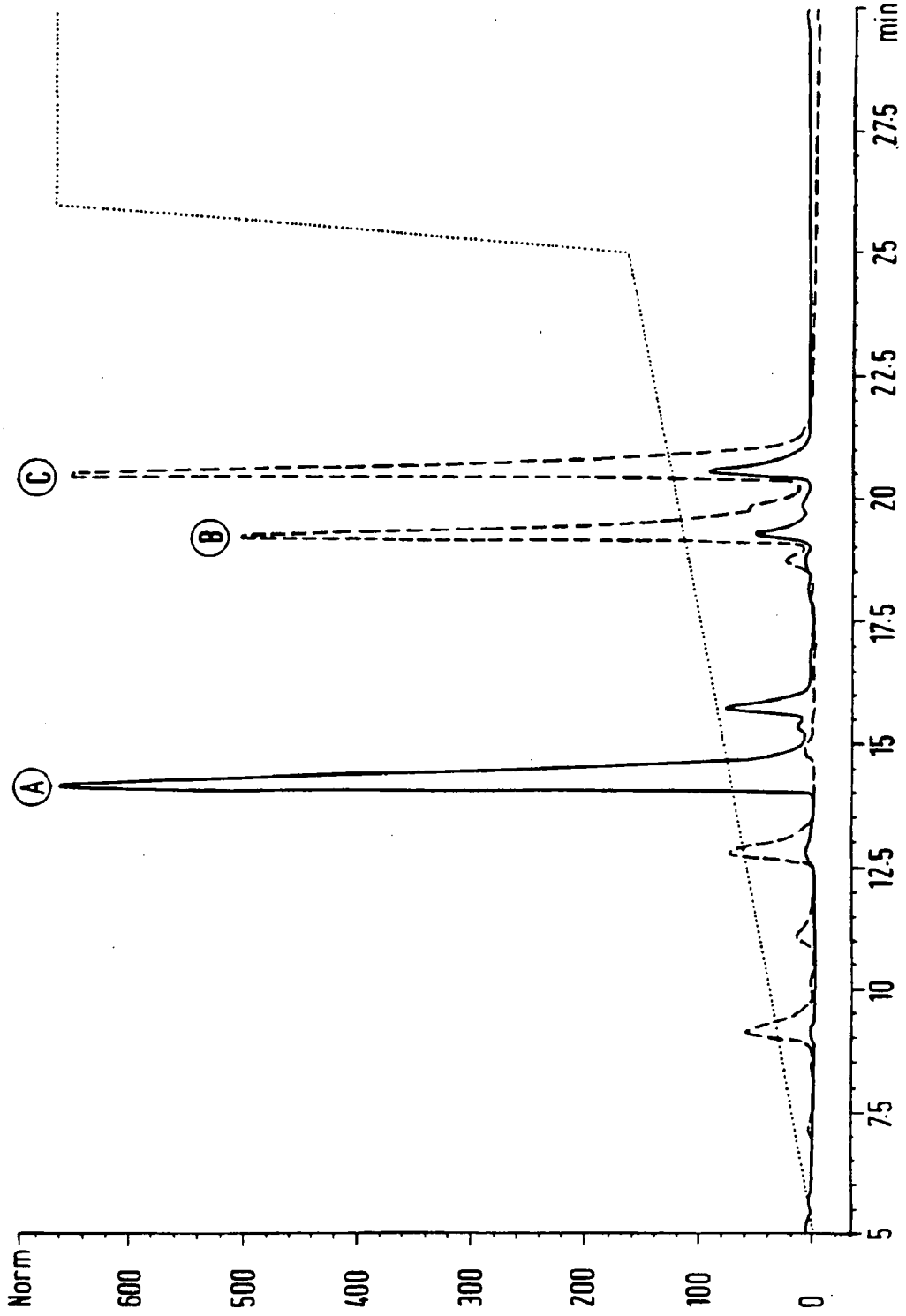
Norm
600
500
400
300
200
100
0
5
7.5
10
12.5
15
17.5
20
22.5
25
27.5
min
A
B
C

FIG.1

INTERNATIONAL SEARCH REPORT

Inter ional application No.
PCT/GB 97/02627

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/62, C07K 7/06, A61K 38/28
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REG, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Science, Volume 277, July 1997, Y. Ido, et al, "Prevention of Vascular and Neural Dysfunction in Diabetic Rats by C-Peptide" page 563 --	1-15
X	Chem.Ber., Volume 106, 1973, Rolf Geiger et al, "Syntheseplan und Darstellung der Sequenz 28-31 des Human-Proinsulin-C-Peptids", page 188 - page 192, see table 1 --	1-5
X	WO 9605309 A2 (THE ROCKEFELLER UNIVERSITY), 22 February 1996 (22.02.96), see seq. 21, claim 4 --	1-4,7

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 January 1998	1 1. 03. 98
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Authorized officer CAROLINA GÓMEZ LAGERLÖF

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/02627

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3953418 A (NOBORU YANAIHARA ET AL), 27 April 1976 (27.04.76), see claim 5	1-5
A	EP 0171887 A2 (ELI LILLY AND COMPANY), 19 February 1986 (19.02.86)	1-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/02627

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 16
because they relate to subject matter not required to be searched by this Authority, namely:
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/12/97

International application No.
PCT/GB 97/02627

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